Supporting Information

An AIE-active tetra-aryl imidazole-derived chemodosimeter for turn-on recognition of hydrazine and its bioimaging in living cells

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1. General methods

Aniline, terephthalaldehyde, acetic acid, p-Anisil, ammonium acetate, malonitrile, ethanol, deionized water, N_2H_4, Cys, ClO^- and other analytical reagents were all purchased from Innochem Technology Co., Ltd.

^1H and ^{13}C NMR spectra were measured with a Bruker AVB-600 spectrometer and Bruker AVB-600 spectrometer, TMS was used as the internal reference, and LC-MS spectra were measured with a Waterse2695 spectrometer. Fluorescence spectra were recorded by an F7000 spectrofluorimeter from Hitachi PharmaSpec. Fluorescence imaging of N_2H_4 in HeLa cells was recorded on a Nikon A1R$^+$ (Japan) laser scanning confocal microscope.

SWJT-31 was dissolved in DMSO to prepare 1.0 mM stock solution, N_2H_4 and other other analytical such as: triethylamine, aniline, n-butylamine, NH_4^+, Na^+, K^+, Mg^{2+}, Zn^{2+}, Cys, Hcy, GSH, ClO^-, HCO_3^-, NO_2^-, SO_4^{2-}, Br^-, S^{2-}, urea, H_2O_2, $ONOO^-$, Ser, Trp, Leu, Glu were dissolved in distilled water to prepare 10.0 mM stock solution. Ultraviolet-visible absorption and fluorescence spectra were recorded after 20.0 μL of **SWJT-31** stock solution and 20.0 μL of N_2H_4 stock solution were diluted to 2.0 mL with DMSO/HEPES (6: 4, v/v, pH = 7.4） buffer solution and incubated at 37℃ for 30 min. The selectivity for the N_2H_4 is investigated by adding other analytical reagents solutions instead of N_2H_4 in a similar way. For fluorescence spectra of AIE effect test, the excitation was set at 420 nm, and the excitation and emission gaps were 10/5 nm. However, fluorescence spectra for detecting hydrazine hydrate, the excitation was set at 340 nm, and the excitation and emission gaps were 5/2.5 nm.

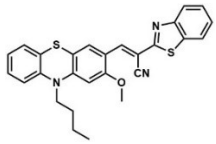
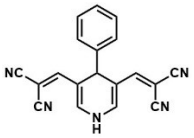
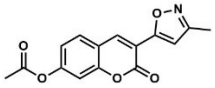
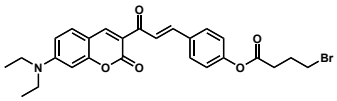
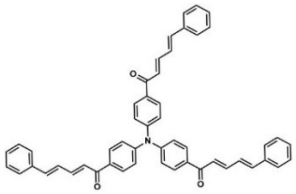
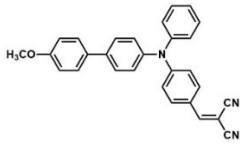
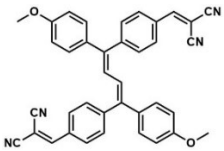
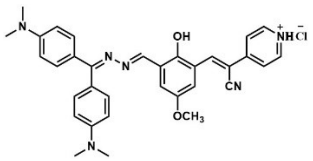
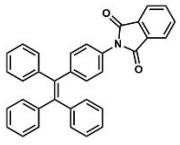
HeLa cells (human cervical cancer cells) were plated in 96-well plates and incubated in a 5% carbon dioxide, 37℃ incubators for 24h. After removing the medium, add probes of different concentrations (0, 5.0 μM, 10.0 μM, 15.0 *M*m, 20.0 μM) to the fresh medium for 24 hours in an incubator. Then, 10.0 μL of MTT (5 mg/mL in PBS, pH 7.4)

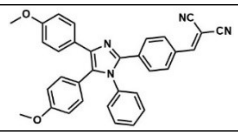
was added to each well, and after the cells were incubated for another 2 hours, the culture supernatant was removed, and the resulting formazan crystals were dissolved in 150 μL of dimethyl sulfoxide. Next, the plates were incubated for an additional 10 minutes at 37℃ on a shaker at 60-70 rpm. Finally, the absorbance (A) of each well at 570 nm was measured using a microplate reader. (Cell relative viability = Asample / Acontrol*100%).

HeLa cells were plated in glass bottom dishes with fresh medium and incubated in a 37℃ incubator for 24 hours. After washing with PBS buffer, fresh medium and 10.0 μM probe were added and incubated for 30 min. After washing three times with PBS buffer, the medium and 40.0 μM N_2H_4 were added for treatment for 30 min. Finally, after washing three times with PBS buffer, the dishes were placed on a confocal laser scanning microscope for cell imaging.

2. Summary of AIE-based fluorescent probes for hydrazine.

Table S1. The comparison of the reported AIE-based hydrazine fluorescent probes.

probes	Solvent system (v/v)	LOD	Response time	reference
	DMSO/PBS (9/1)	0.11 nM	-	30
	DMSO/PBS (2/3)	11 nM	60 min	31
	CH_3CN/H_2O (3/7)	2.90 ppb	45 min	32
	DMSO/PBS (6/4)	0.04 μM	25 min	33
	THF/H_2O (9:1)	0.119 nM	-	34
	DMSO/tris-HCl (3/1)	0.196 μM	20 min	35
	DMSO/PBS (9/1)	2.88 ppb	3 min	36
	DMSO/H_2O (1/99)	1.2 ppb	2 min	37
	CH_3CN/HEPES (3/97)	6.4 ppb	-	38

	DMSO/HEPES (6/4)	33.8 nM	30 min	This work

3. Synthesis of the M-CHO and SWJT-31.

Q-CHO was synthesized according to previous work[36]. Aniline (93 mg, 1.0 mmol) and terephthalaldehyde (134 mg, 1.0 mmol) were dissolved in acetic acid (15 mL) and reacted at room temperature. After 1 h, p–Anisil (270 mg, 1.0 mmol) and ammonium acetate (539 mg, 7.0 mmol) were added to the reaction flask to react at 120 ℃ for 10 h to obtain **Q-CHO**.

The synthetic procedures of **SWJT-31** were shown in Scheme 2. **Q-CHO** (92 mg, 0.2 mmol) and malononitrile (12.6 μL, 0.2 mmol) were dissolved in anhydrous ethanol (2.0 mL), followed by piperidine (8.0 μL). Next, the mixture was stirred at room temperature overnight, and the orange yellow precipitate was precipitated and then filtered. After precipitation and purification, the orange yellow powder **SWJT-31** (71 mg) was obtained with a yield of 69.8%.^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.76 (d, J = 8.6 Hz, 2H), 7.66 (s, 1H), 7.59 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.39–7.33 (m, 3H), 7.10–7.03 (m, 4H), 6.83–6.76 (m, 4H), 3.79(s, 3H), 3.77 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ =159.5, 158.8, 158.7, 144.1, 139.2, 136.9, 136.4, 132.3(2C), 131.8, 130.7(2C), 129.9, 129.5(2C), 129.0, 128.9(2C), 128.4(2C), 128.3(2C), 126.7, 122.1, 114.0(2C), 113.9, 113.7(2C), 112.7, 81.9, 55.2, 55.1 ppm. LC-MS: *m/z* 475.2 $[M + H]^+$.

4. ^{1}H, ^{13}C NMR spectra and LC-MS of SWJT-31.

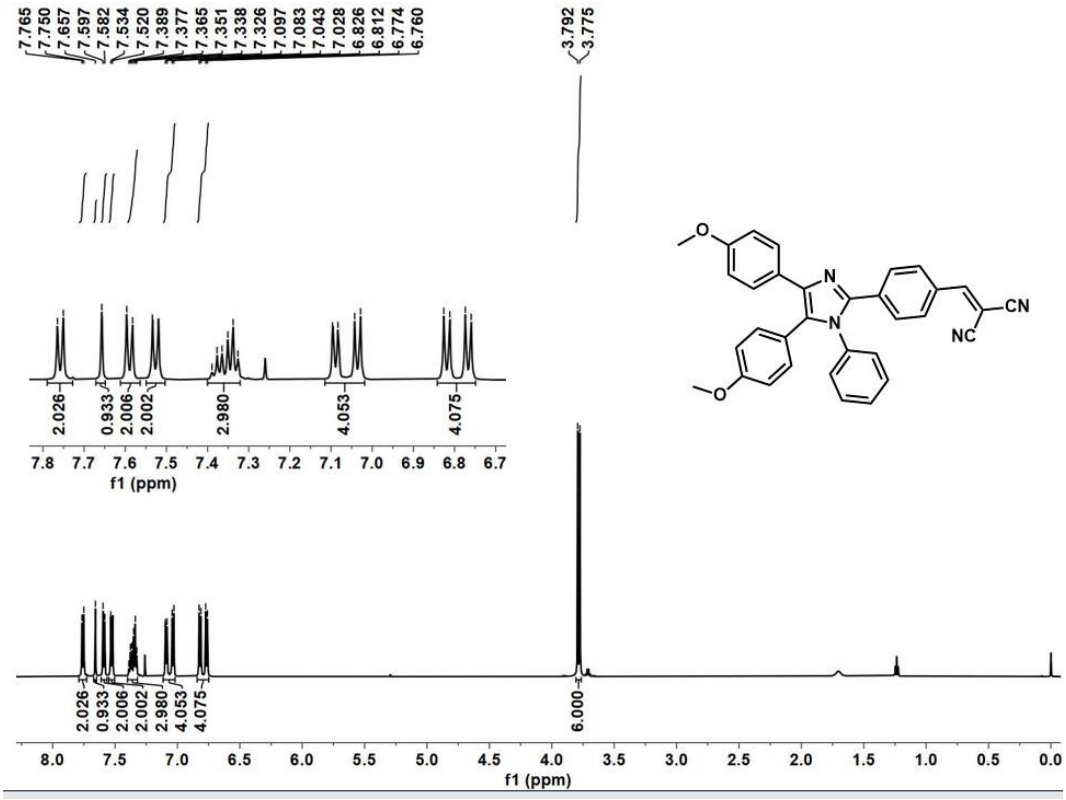


Figure S1. ^{1}H NMR spectrum of **SWJT-31** in $CDCl_3$.

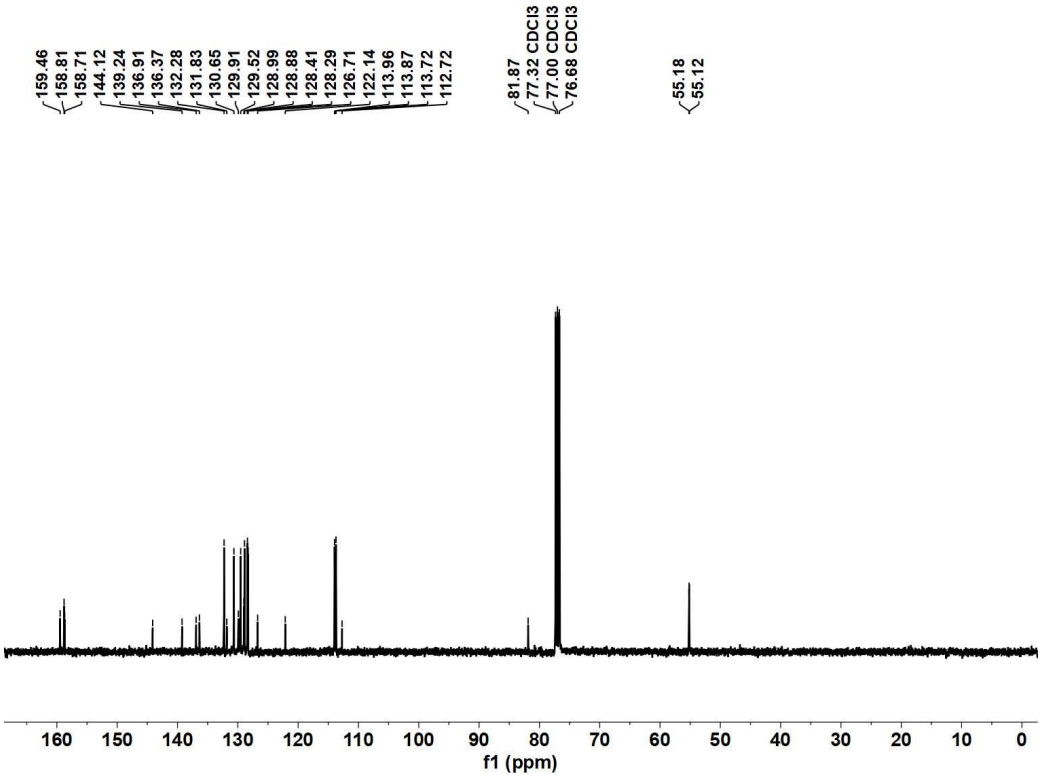


Figure S2. ^{13}C NMR spectrum of **SWJT-31** in $CDCl_3$.

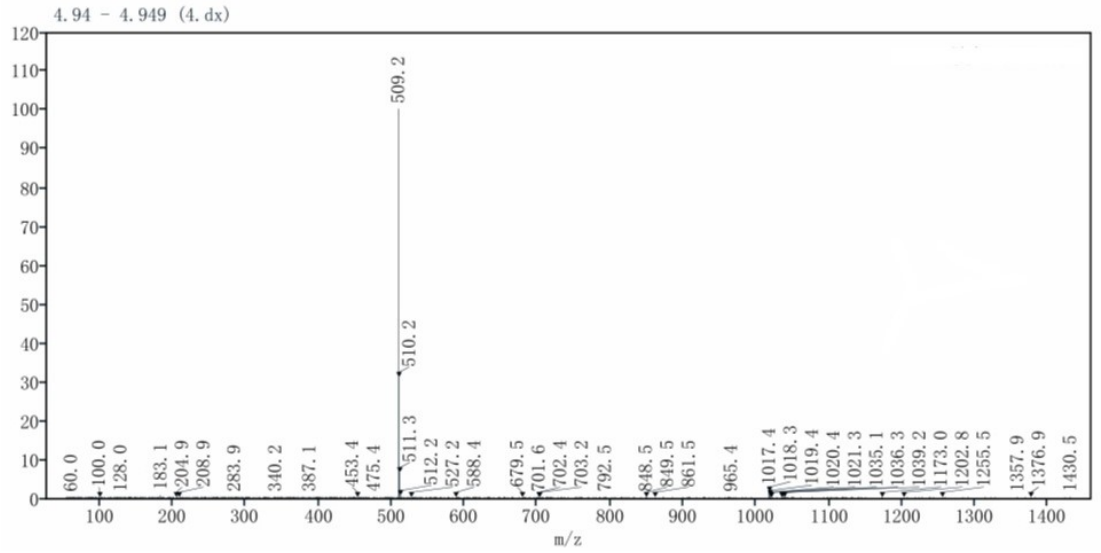


Figure S3. LC-MS spectrum of **SWJT-31**.

5. Basic properties of SWJT-31.

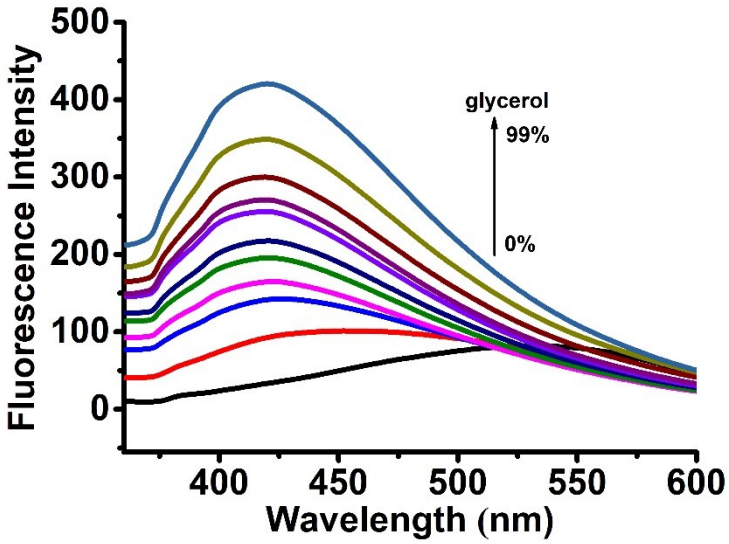


Figure S4. Fluorescence spectra of **SWJT-31** (10.0 μM) in the presence of different viscosity in glycerol/ DMSO fraction.

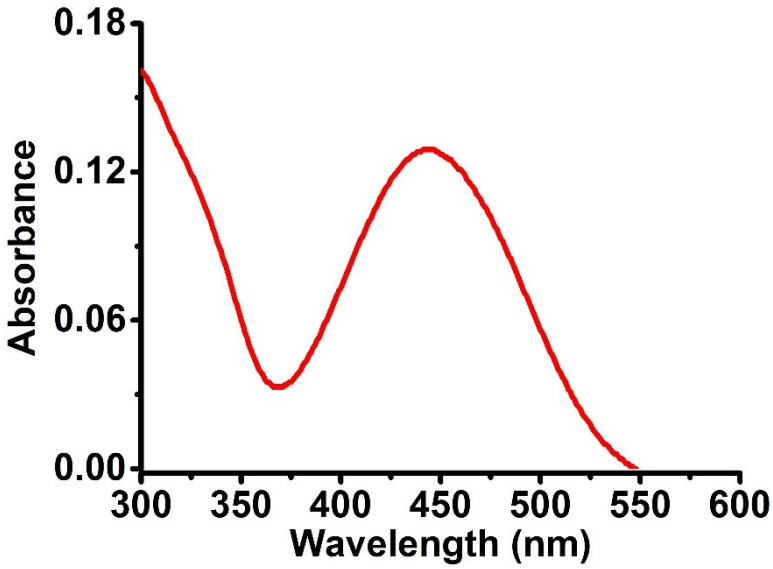


Figure S5. In DMSO/HEPES (v/v=1:99, pH = 7.4) buffer solution, absorption spectra of **SWJT-31** (10.0 μM).

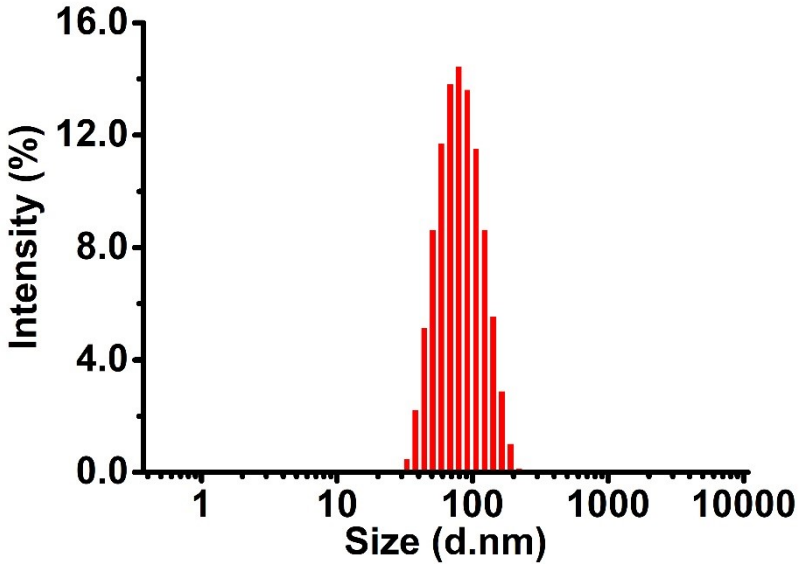


Figure S6. Size distribution of **SWJT-31** in the aggregated state in 99% HEPES buffer solution (pH = 7.4).

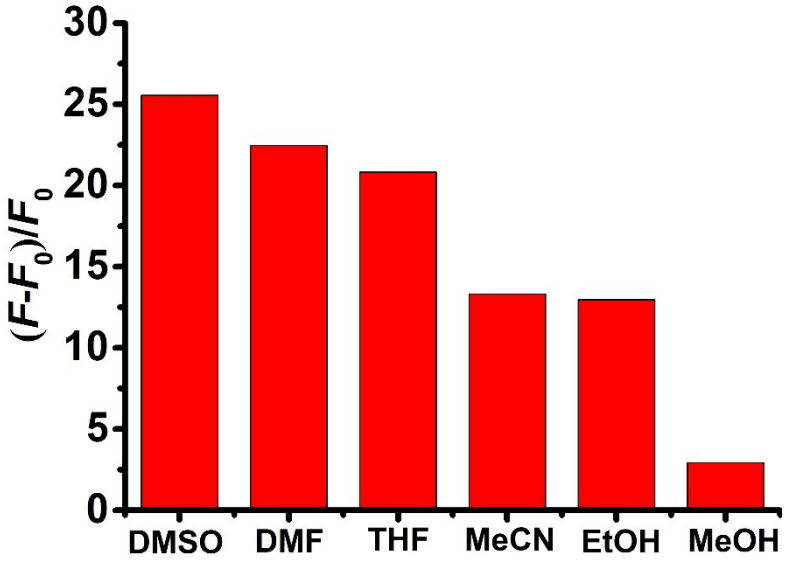


Figure S7. The fluorescence enhancement times of **SWJT-31** (10.0 μM) and **SWJT-31**+ N_2H_4 (100.0 μM) in different organic phases at 497nm (λ_{ex} = 340 nm).

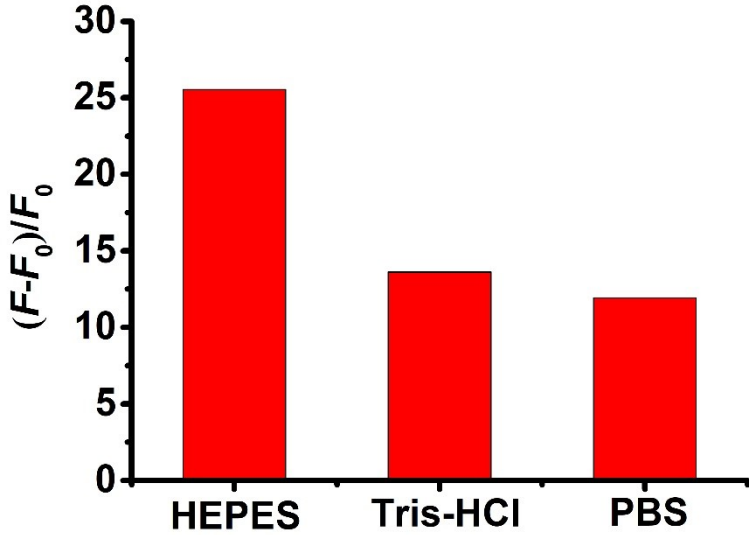


Figure S8. The fluorescence enhancement times of **SWJT-31** (10.0 μM) and **SWJT-31**+ N_2H_4 (100.0 μM) in different organic phases at 497nm (λ_{ex} = 340 nm).

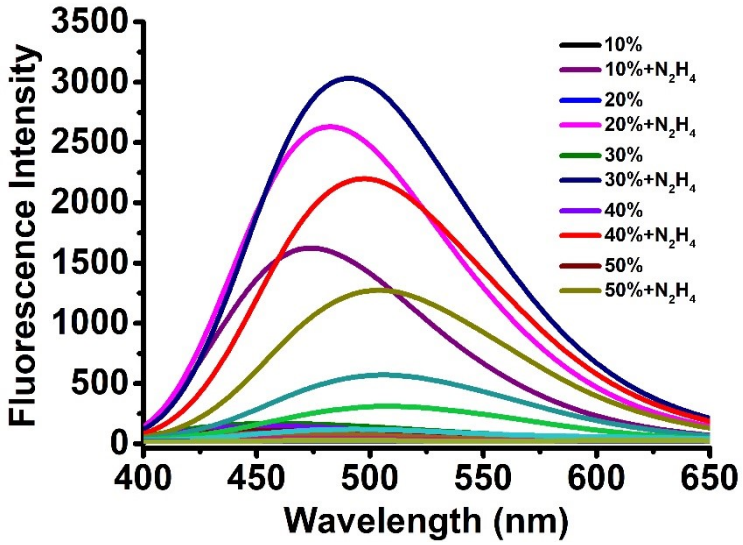


Figure S9. Fluorescence spectra versus the content of the DMSO/HEPES mixture of **SWJT-31** in the presence of N_2H_4(100.0 μM) (λ_{ex} = 340 nm).

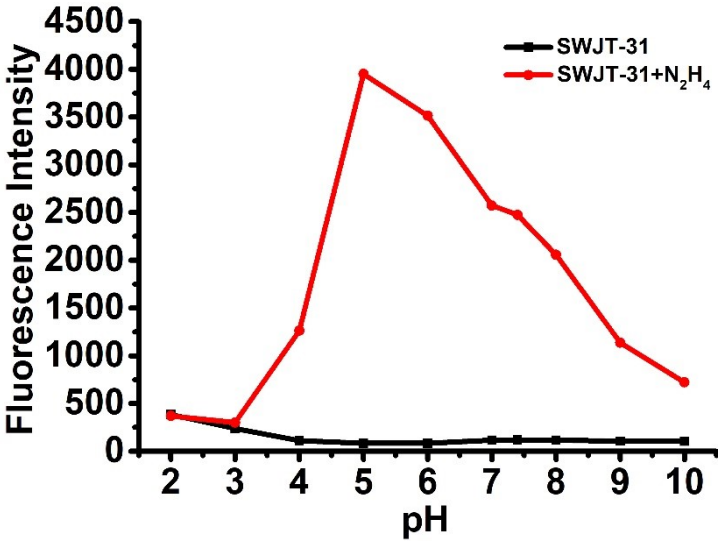


Figure S10. Effects of pH on the reaction of **SWJT-31** (10.0 μM) with N_2H_4 (100.0 μM) (λ_{ex} = 340 nm).

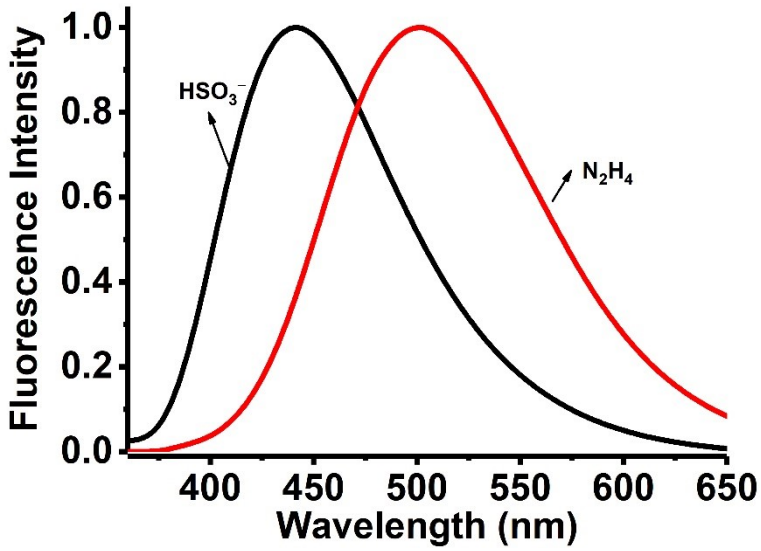


Figure S11. Fluorescence spectra of **SWJT-31**+ N_2H_4 and **SWJT-31**+ SO_3^{2-}.

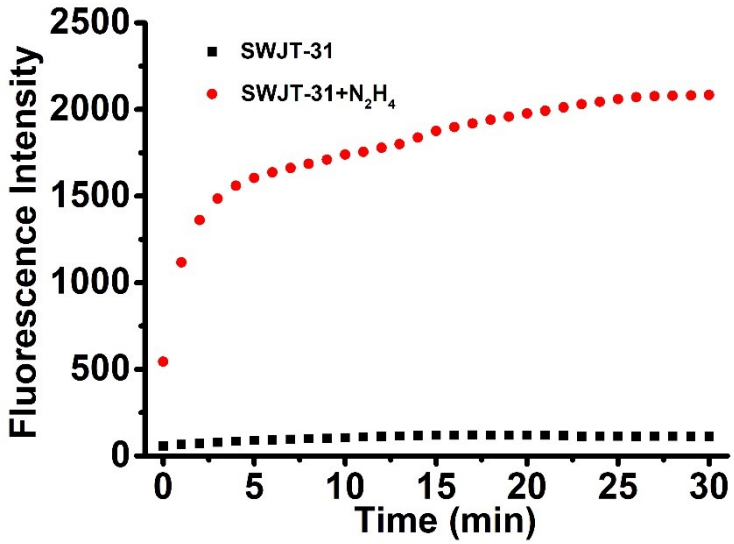


Figure S12. In the absence or presence of N_2H_4 (10.0 μM), time-dependent fluorescence intensity of **SWJT-31** (10.0 μM) in DMSO: HEPES (6:4, v/v, pH = 7.4) buffer solution at 497 nm (λ_{ex} = 340 nm).

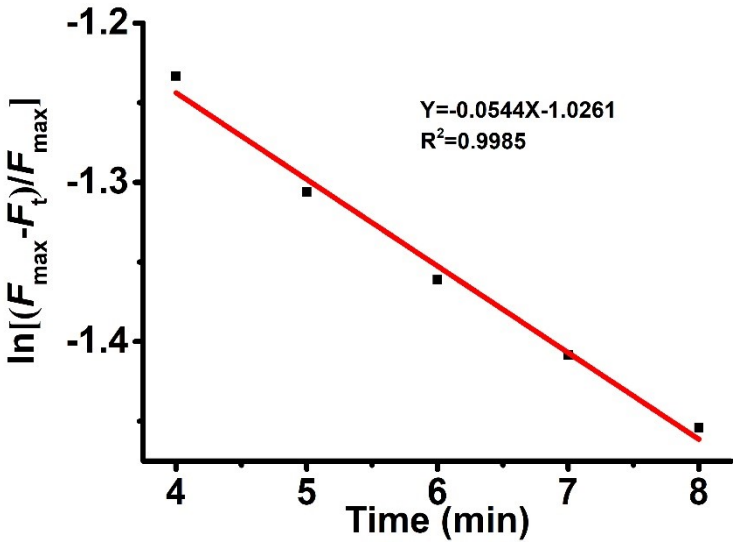


Figure S13. Pseudo first-order kinetic plots of **SWJT-31** (10.0 μM) with the addition of N_2H_4 in HEPES buffer solution (60% DMSO, pH = 7.4) (λ_{ex} = 340 nm).

The result of the analysis as follows：

$$\ln [(F_{max}-F_t) / (F_{max})] = -k_{obst}$$

$$t_{1/2} = \ln 2/k_{obst}$$

Where F_{max} and F_t are the fluorescent intensity at maximum emission wavelength, and time t. k_{obs} is the pseudo-first-order rate constant.

$$k_{obs} = 9.06 \times 10^{-4}\ s^{-1}$$

$$t_{1/2} = 12.74\ min$$

6. Application of SWJT-31 as test strips.

Table S2. Determination of N_2H_4 in water samples.

Sample	spiked(μM)	Found mean ± SD[a] (μM)	Recovery
Tape water	0	Not Detected	
	2	1.854±0.027	92.69%
	6	5.669±0.115	94.49%
	10	10.736±0.029	107.4%
Spring water	0	Not Detected	
	2	1.916±0.012	95.81%
	6	6.259±0.027	104.3%
	10	10.17±0.239	101.7%
Jing Lake	0	Not Detected	
	2	1.887±0.043	94.37%
	6	5.214±0.106	86.90%
	10	9.724±0.106	97.24%

7. ^{1}H, ^{13}C NMR spectra and LC-MS of the product SWJT-N2.

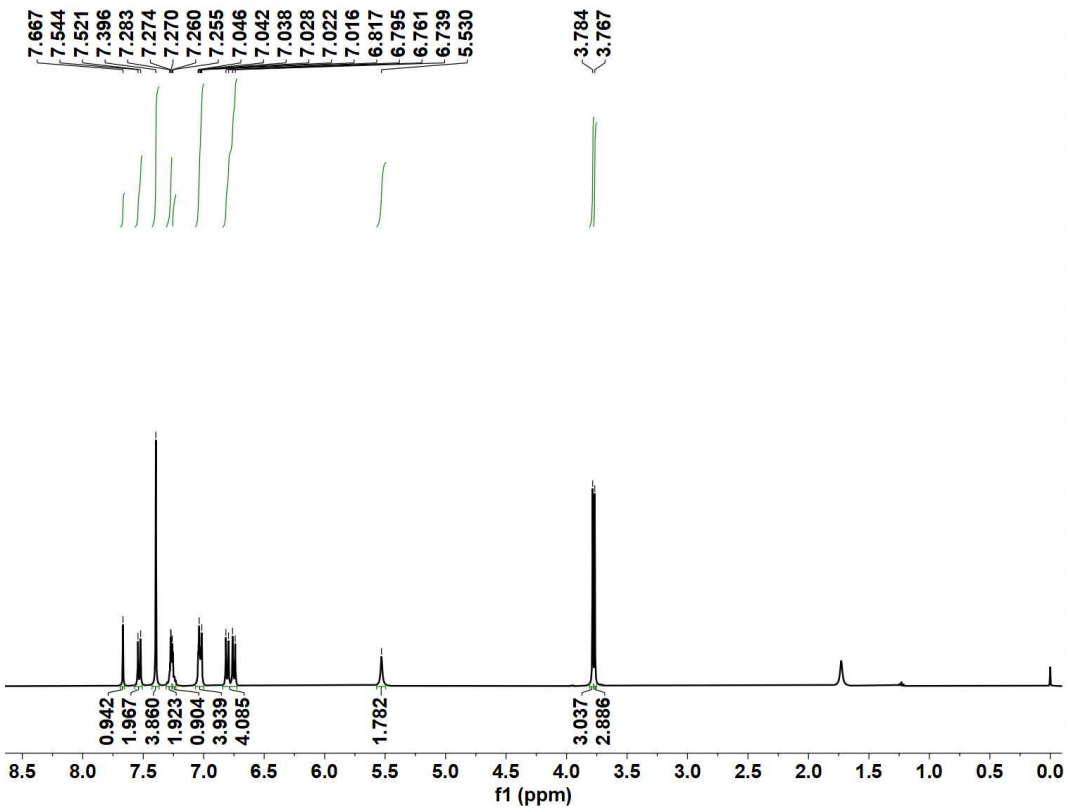


Figure S14. ^{1}H NMR spectrum of the product **SWJT-N2** in $CDCl_3$.

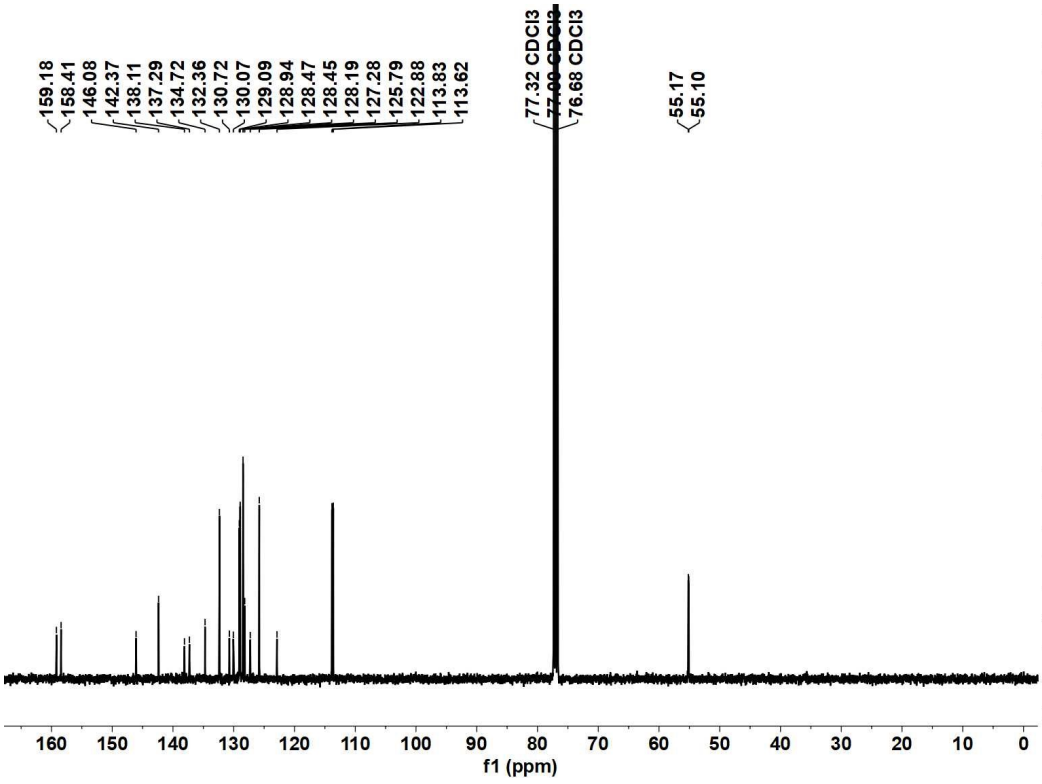


Figure S15. ^{13}C NMR spectrum of the product **SWJT-N2** in $CDCl_3$.

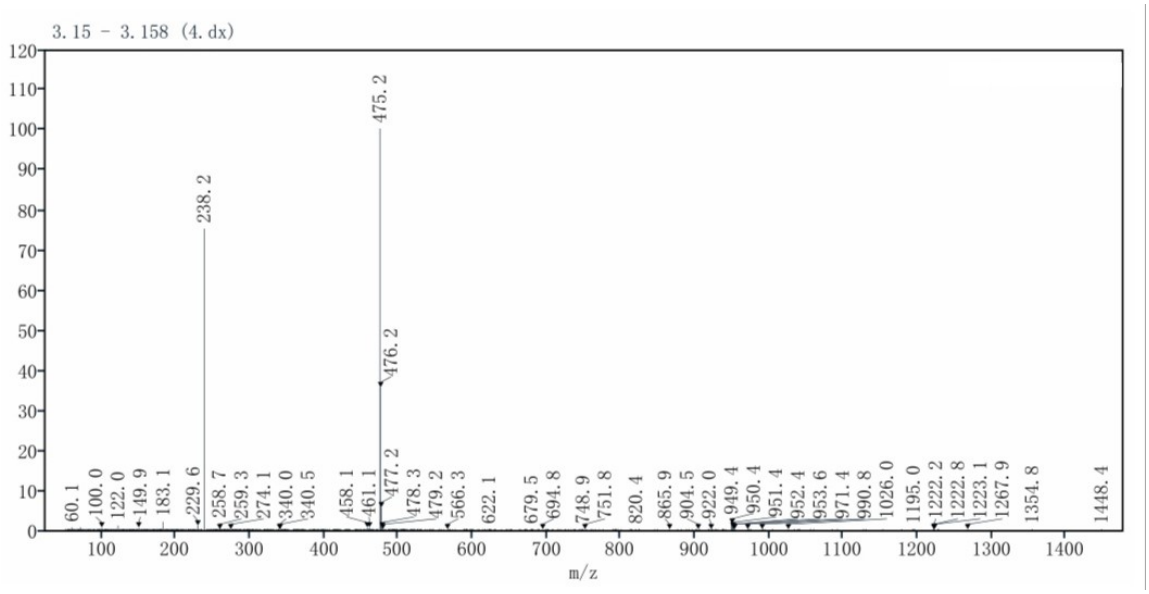


Figure S16. LC-MS spectrum of the product **SWJT-N2**.

8. Cytotoxicity of SWJT-31 in living HeLa cells.

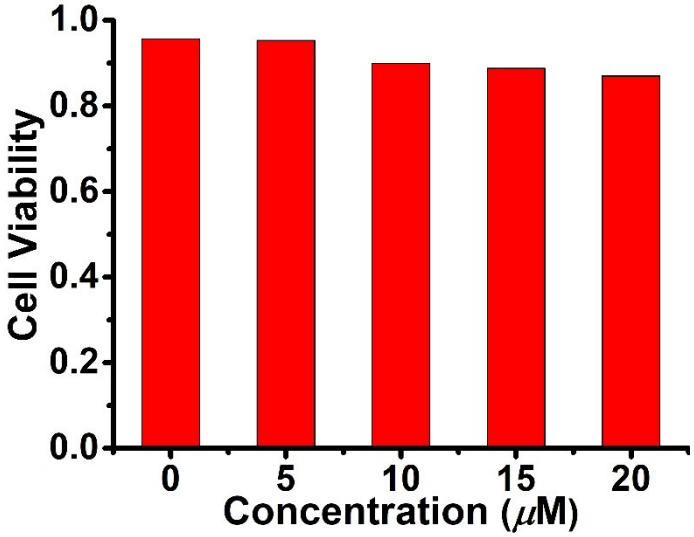


Figure S17. The viability of HeLa cells was determined by MTT assay after incubation with different concentrations of **SWJT-31** for 24 h.